Best /	Avaj	е Сору
--------	------	--------

# PHOCESSED BY

F.

Application Number

O8/357, 745

Group Art Unit Examiner

Paper No. 21

Assistant Commissioner for Pietents Washington, DC 20231

Application No	on pageon pageof an application that is open to filed
inspection, i.e., Application No.  D) an application in which the applicant has filed an auth application to the public.	enzation to lay open the compi
application to the public.	•
ise direct any correspondence concerning this request to	the following address:
	2 2
Signature	3-29-98 Date
Typed or printed name	FOR PTO USE ONLY
Typed of Griffied Harrie	Approved by:



# United States Patent [19

Weiss et al.

[11] Patent Number:

5,851,832

[45] Date of Patent:

Dec. 22, 1998

# [54] IN VITRO GROWTH AND PROLIFERATION OF MULTIPOTENT NEURAL STEM CELLS AND THEIR PROGENY

[75] Inventors: Samuel Weiss; Brent Reynolds, both of Alberta, Canada; Joseph P.

Hammang; E. Edward Baetge, both of

Barrington, R.I.

[73] Assignee: Neurospheres, Ltd., Canada

[21] Appl. No.: 486,648

[22] Filed: Jun. 7, 1995

# Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 270,412, Jul. 5, 1994, abandoned, which is a continuation of Ser. No. 726,812, Jul. 8, 1991, abandoned, and a continuation-in-part of Ser. No. 385,404, Feb. 7, 1995, abandoned, which is a continuation of Ser. No. 961,813, Oct. 16, 1992, abandoned, which is a continuation-in-part of Ser. No. 726,812, and Ser. No. 359, 945, Dec. 20, 1994, abandoned, which is a continuation of Ser. No. 221,655, Apr. 1, 1994, abandoned, which is a continuation of Ser. No. 967,622, Oct. 28, 1992, abandoned, which is a continuation-in-part of Ser. No. 726,812, Jul. 8, 1991, abandoned, and Ser. No. 376,062, Jan. 20, 1995, abandoned, which is a continuation of Ser. No. 10,829, Jan. 29, 1993, abandoned, which is a continuation-in-part of Ser. No. 726,812, and Ser. No. 11,099, Sep. 23, 1994, abandoned, which is a continuation-in-part of Ser. No. 311,099, Sep. 23, 1994, abandoned, which is a continuation-in-part of Ser. No. 326,812, and Ser. No. 318, 730, Nov. 14, 1994, abandoned, which is a continuation-in-part of Ser. No. 726,812, and Ser. No. 726,812.

[51]	Int. Cl. <sup>6</sup>	C12N 5/06; C12N 5/08;
		C12N 5/02
[52]	U.S. Cl	
		435/383; 435/384
[58]	Field of Search	435/240.2, 325,

# [56] References Cited

#### **U.S. PATENT DOCUMENTS**

435/366, 368, 377, 383, 384

4,753,635	6/1988	Sagen ét al 604/49
4,980,174	12/1990	Sagen et al 424/563
5,082,670	1/1992	Gage 424/520
		Lee et al 435/172.3
5,411,883	5/1995	Boss et al 435/29
5,612,211	3/1997	Wilson et al 435/378

#### FOREIGN PATENT DOCUMENTS

0 233 838	8/1987	European Pat. Off
89/03872	5/1989	WIPO .
90/06757	6/1990	WIPO .
91/02003	2/1991	WIPO .
91/09936	7/1991	WIPO .
91/17242	11/1991	WIPO .
93/01275	1/1993	WIPO .
93/09802	5/1993	WIPO .
94/03199	2/1994	WIPO .

# OTHER PUBLICATIONS

Almazan et al., "Epidermal Growth and Bovine Growth Hormone Stimulate Differentiation and Myelination of Brain Cell Aggregates in Culture," *Developmental Brain Research*, 21:257–264 (1985).

Anchan et al., "Trophic Factors Influence Proliferation of Germinal Neuroepithelial Cells of the Retina," J. Cell Biol., 109:58a, Abstract No. 308 (1989).

Anchan et al., "EGF and TGF- $\alpha$  Stimulate Retinal Neuroepithelial Cell Proliferation in Vitro," *Neuron*, 6(6):923-936 (1991).

Bayer et al., "Neuron production in the Hippocampus and olfactory bulb of the adult rat Brain: addition or replacement?", Annals NY. Acad. Sci. 457:163-172 (1985).

Björklund et al., "Neural Grafting in Animal Models of Neurodegenerative Diseases," *Ann. New York Acad. Sci.*, 457:53–81 (1985).

Bouvier et al., "Basic Fibroblast Growth Factor (bFGF) Promotes the Survival and Proliferation of Mesencephalic Neuronal Precursors in Vitro," *Society for Neuroscience Abstracts*, vol. 18, Abstract No.: 403.7 (1992).

Boyles et al., "Accumulation of Apolipoproteins in the Regenerating and Remyelinating Mammalian Peripheral Nerve," J. Biol. Chem., 265(29):17805-17815 (1990).

Calof et al., "Analysis of Neurogenesis in a Mammalian Neuroepithelium: Proliferation and Differentiation of an Olfactory Neuron Precursor in Vitro," *Neuron*, 3:115–127 (1989).

Cattaneo et al., "Identifying and Manipulating neuronal stem cells," TINS, 14(8): 338-340 (1991).

Cattaneo et al., "Proliferation and differentiation of neuronal stem cells regulated by nerve growth factor," *Nature*, 347:762–765 (1990).

Cepko "Immortalization of neural cells via retrovirus-mediated oncogene transduction," Ann. Rev. Neurosci., 12:47-65 (1989).

Deloulme et al., "Establishment of Pure Neuronal Cultures From Fetal Rat Spinal Cord and Proliferation of the Neuronal Precursor Cells in the Presence of Fibroblast Growth Factor," *Journal of Neuroscience Research*, 29:499–509 (1991).

Dunnett et al., "Dopamine-rich transplants in experimental Parkinsonism," TINS, 266-270 (Jul. 1983).

Emerich et al., "Behavioral Effects of Neural Transplantation," Cell Transplantation, 1:1-27 (1992).

Faaland et al., "Rapid uptake of tyrphostin into A431 human epidermoid cells is followed by delayed inhibition of epidermal growth factor (EGF)-stimulated EGF receptor tyrosine kinase activity", Mol. Cell Biol. 11(5):2697-2703 (1991).

(List continued on next page.)

Primary Examiner—George C. Elliott
Assistant Examiner—Johnny F. Railey, II
Attorney, Agent, or Firm—Flehr Hohbach Test Albrition &
Herbert LLP

# [57] ABSTRACT

A method for the in vitro proliferation and differentiation of neural stem cells and stem cell progeny comprising the steps of (a) isolating the cells from a mammal, (b) exposing the cells to a culture medium containing a growth factor, (c) inducing the cells to proliferate, and (d) inducing the cells to differentiate is provided.

# 80 Claims, 3 Drawing Sheets